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A Probable Endocrine Basis for the Depression of Ketone Bodies during Infectious or Inflammatory State in Rats*

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ABSTRACT. The effects of infection with *Streptococcus pneumoniae, Francisella tularensis,* and Venezuelan equine encephalitis virus as well as inflammatory stress induced by the administration of turpentine and endotoxin on plasma ketone bodies and insulin were studied in white rats. All of the infectious/inflammatory stresses caused a significant decrease in the ketonemia of fasting and an elevation of plasma insulin. When a pneumococcal infection was initiated in a diabetic rat, inhibition of fasting ketonemia did not occur. Similarly, pneumococcal infection in the hypophysectomized rat did not result in a noticeable depression of either fasting ketonemia or plasma FFA. The increase in circulating insulin appears to be closely correlated with the inhibition of fasting ketonemia noted in the infectious/inflammatory stress. (*Endocrinology* **107**: 596, 1980)

WHEN induced experimentally in fasted rats, inflammatory processes of many different causes are accompanied by an inhibition of the ketonemia associated with fasting and a depression in the concentration of plasma FFA (1–6).

An increase in plasma insulin and glucagon values during these diseased states has been noted in man and experimental animals by many investigators (3, 4, 7–13). The inhibition of ketonemia noted during infection in man has been hypothesized to be secondary to an insulin-induced decrease in the mobilization of FFA from adipose tissue (11). However, preliminary data from this laboratory suggest that the rate of hepatic ketogenesis is diminished during inflammatory/infectious states despite the availability of excess exogenous FFA supplied to the liver (14). Since many tissues, principally skeletal and cardiac muscle and brain, are able to use ketone bodies as a source of energy (15, 16), a decrease in the availability of circulating ketone bodies increases the dependence on other sources of energy when cellular metabolism is accelerated during periods of fever. This need is met principally by gluconeogenesis employing amino acids derived from protein of skeletal muscle and other peripheral body tissues. As a result, a large or generalized inflammatory process is also characterized by an increased loss of body nitrogen. It appears that the infected host is not able to use some of the major mechanisms normally called into play during fasting to conserve body protein (17–19).

This paper presents data which indicate that the inhibition of the fasting ketonemia which accompanies infection/inflammation may be modulated by endocrine responses. The new data support the importance of insulin as a key antiketonemic factor during infection/inflammation; the insulin response may, in turn, be influenced by the hypophysis.

Materials and Methods

Animals used were male or female rats (Fisher-Dunning, F-344/Mai f, Microbiological Associates, Walkersville, MD), weighing 150–200 g. Rats were maintained on a commercial diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) until the beginning of an experiment and were housed in rooms maintained at 23 ± 1 C.

For other studies, hypophysectomized male rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 100–150 g, were maintained for 2 weeks in the environment described above until it was ascertained that there was no weight gain. Adrenalectomized rats (Charles River Breeding Laboratories), weighing 100–150 g, were maintained with physiological saline.

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* In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Appropriate normal control and thyroidectomized rats (150–160 g) were also obtained from Charles River. These animals were used within 3 weeks of arrival.

Inflammatory models

Rats were inoculated sc with 10^4 virulent, colony-forming units (CFU) of *Streptococcus pneumoniae* Ia5 in the nape of the neck (1). Other groups of rats were injected ip with 10^8 CFU *Francisella tularensis*, live vaccine strain (USAMRIID strain) (20). Another group of rats was sc given $10^{4.3}$ plaque-forming units (PFU) of the V-198 strain of a rat virulent Venezuelan equine encephalitis (VEE) (2). Controls for these groups received an equal number of heat-killed bacteria or virus.

In still other groups, sterile inflammatory abscesses were produced by sc inoculation with 1 ml turpentine (Phipps Production Corp., Boston, MA) into the nape of the neck. Another stress was induced by the ip injection of 1.0 mg (1 ml) endotoxin, lipopolysaccharide W, *Escherichia coli* 01271 BS (Difco Laboratories, Detroit, MI) diluted in physiological saline. Controls for the latter two groups were given 1 ml physiological saline by the same respective routes. In most experiments, food was withheld from rats for 24 h before inoculation and thereafter. In all experiments, a sufficient number of rats was inoculated so that a minimum of six rats could be killed at each experimental time point. The response to the infectious or inflammatory stress was characterized by a rise in rectal temperature and a depression of plasma zinc (21).

Blood was obtained from anesthetized rats (halothane chamber) by opening the chest cavity, cutting the vena cava, and collecting blood in heparinized tubes for the preparation of plasma.

Diabetic rats

Rats were made diabetic by the iv injection via the penile vein of 0.1 ml streptozotocin (Sigma Chemical Co., St. Louis, MO) at a dosage of 100 mg/kg. Rats were lightly anesthetized with halothane during the administration of the streptozotocin. Control rats received injections of equal volumes of sterile saline. For the first 24 h after the administration of streptozotocin, the rats were maintained on 5% glucose in their drinking water.

After 24 h, rats which had received the streptozotocin demonstrated significant ketonemia and high blood glucose values (500–600 mg/dl). The 5% dietary glucose was then replaced with water and 2 U insulin (Isophane Insulin Suspension, USP, zinc insulin crystals, Eli Lilly Co., Indianapolis, IN) were administered im. This amount of insulin caused a reduction in the values of plasma ketone bodies and glucose and was maintained until plasma ketone concentration increased again. The rats were subsequently given 3–5 U/day to stabilize the plasma ketone concentration between 1–3 μmol/ml. Half of the diabetic rats were then infected with 10^4 *S. pneumoniae*, and half received an equal quantity of heat-killed bacteria sc. All food was removed, and insulin therapy was discontinued. After 24 h, half of the infected diabetic group and half of the diabetic control group were given 2 U insulin im.

Assay procedures

Published procedures were used for the determination of ketone bodies (22), FFA (23), zinc (21), insulin (8), and glucagon (24). Insulin to glucagon molar ratios were calculated as described by Muller *et al.* (25).

Statistical tests

Significance of group means was determined by Student's *t* test. Stressed rats in each study were compared to their appropriate control group at each selected time interval. Data are presented as the mean ± SEM for at least six rats per group.

Results

A study was initiated to determine whether the unexpected decrease in the plasma values of ketone bodies during the anorexia accompanying inflammatory stress was regulated by endocrine effects. An infectious stress, *i.e.* 10^4 *S. pneumoniae*, was administered to normal, thyroidectomized, and adrenalectomized rats. As shown in Fig. 1, the depression of fasting ketosis was equivalent in all groups, indicating that the thyroid, the adrenal medulla, and the adrenal cortex were not directly involved in this response to inflammatory stress. When this infection was studied in female rats, the suppressed ketogenic response was virtually identical to that seen in male rats (data not shown).

A detailed series of infectious and inflammatory

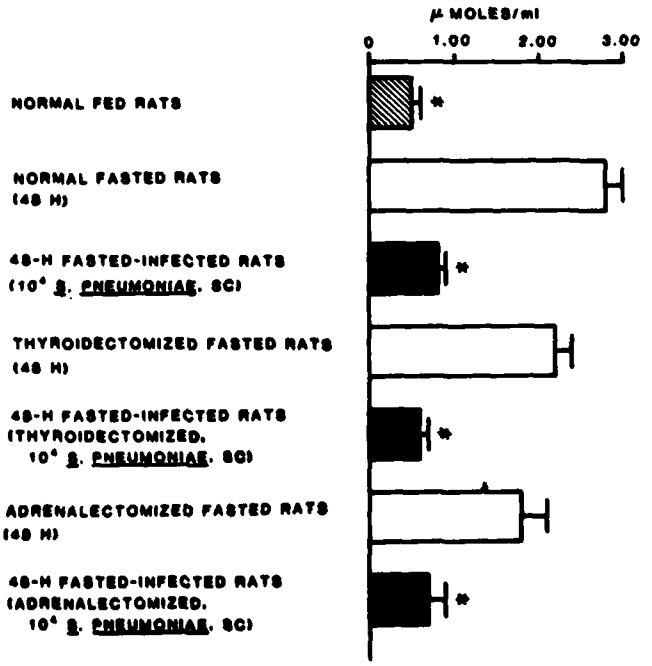

FIG. 1. Effect of fasting and fasting plus infection with *S. pneumoniae* on ketone body production in normal, adrenalectomised, and thyroidectomised rats 48 h post inoculation ($P \leq 0.01$).

stresses was imposed on rats to determine whether the observed depression in plasma ketones during pneumococcal infection was typical of inflammatory stress in general. In all studies (Fig. 2) there was a significant depression in plasma ketone bodies. The same stresses were also accompanied by a marked depression in plasma FFA (800 ± 50 to 300 ± 50 μeq/liter). All of the inflammatory stresses caused a significant elevation in plasma insulin concentrations, as shown in Fig. 3. Curnow *et al.* (9) showed that infection of rats with *S. pneumoniae* caused elevated plasma glucagon values. All of the stresses which we applied to rats also caused plasma glucagon concentrations to increase from 300 ± 50 to 1500 ± 150 pg/ml.

To study further the role of insulin in the observed depression of plasma ketone bodies during inflammatory stress, infection with *S. pneumoniae* was imposed on rats previously made diabetic by the administration of streptozotocin (Fig. 4). When insulin therapy was discontinued in diabetic rats, this infection did not cause marked ketone body depression. Moreover, if a therapeutic dose of insulin was administered to ketotic infected rats or noninfected diabetic rats, ketosis was significantly reduced. Concentrations of plasma FFA in the diabetic rats responded in a fashion similar to that observed for ketone bodies, *i.e.* they increased in the absence of insulin and decreased after insulin.

When fasted hypophysectomized rats were infected with *S. pneumoniae,* there was no depression in either plasma ketone bodies or FFA (Figs. 5 and 6). Moreover, the infected hypophysectomized rat did not demonstrate detectable increases in plasma insulin (Fig. 7). In most instances, the concentration of insulin in the plasma of both infected and noninfected fasted hypophysectomized rats was below the limit of the assay system employed, *i.e.* less than 4 μU/ml. As shown in Fig. 7, plasma insulin

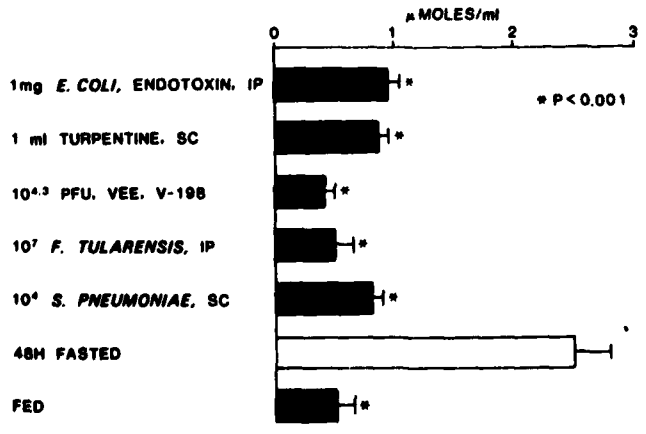

FIG. 2. Effect of infectious and inflammatory stress on plasma ketone body concentrations 48 h post inoculation.

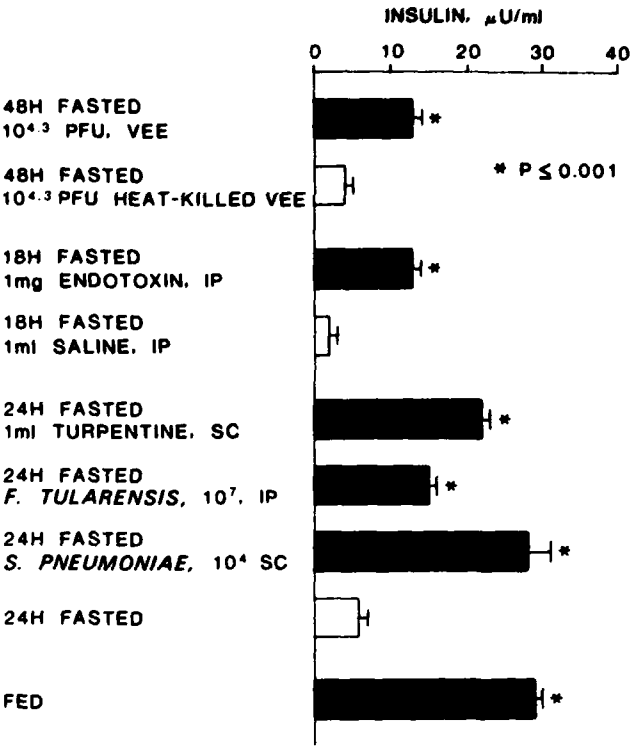

FIG. 3. Effect of infections and inflammatory stress on plasma insulin concentrations. The times indicated are the points of maximal insulin elevation post inoculation.

was elevated in response to the infection in the controls for the hypophysectomized rat study.

Discussion

A wide variety of metabolic changes occurs in a host animal subjected to infectious and/or inflammatory stress (17). Important questions remain as to how an interaction of hormonal regulatory mechanisms may influence these changes. Although anorexia is common to severe generalized and inflammatory states, the hormonal and metabolic responses differ from those seen in simple starvation. Uncomplicated brief starvation is characterized by increased plasma ketone and FFA concentrations, an increased FFA to albumin ratio, decreased plasma insulin concentrations, and decreased insulin to glucagon ratio. In contrast, when infection/inflammation occurs in the rat host experiencing brief starvation, there are markedly different endocrine and metabolic responses. For example, plasma ketone and FFA values decline, but a decline in plasma concentrations causes the FFA to albumin ratio to remain at fasting values; concomitantly, insulin values increase moderately and glucagon values increase markedly, with a resultant decrease in the molar insulin to glucagon ratio from 1.90 to 0.50 compared to that in the noninfected fed

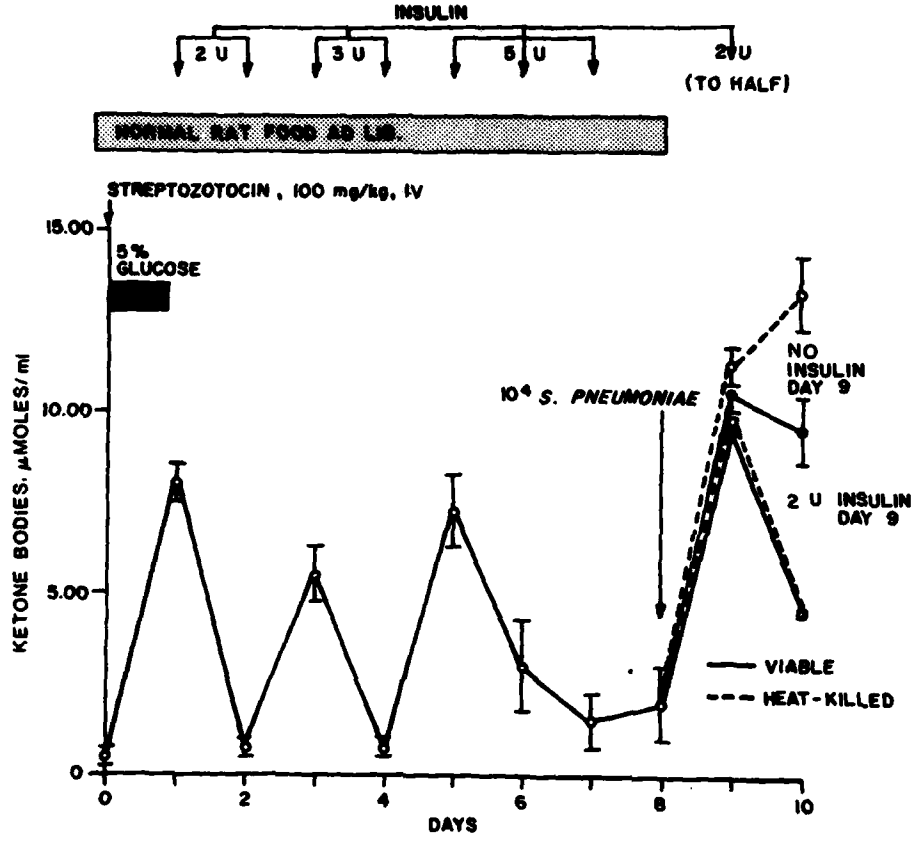

FIG. 4. Effect of infection with *S. pneumoniae* on ketone bodies in diabetic rats. The details of the experiment are described in *Materials and Methods*. ○, Control rats and rats which received 10^4 heat-killed *S. pneumoniae* sc; ●, rats which received 10^4 viable *S. pneumoniae* sc. Control (□) and infected (■) rats which received 2 U insulin im 24 h post inoculation are also shown.

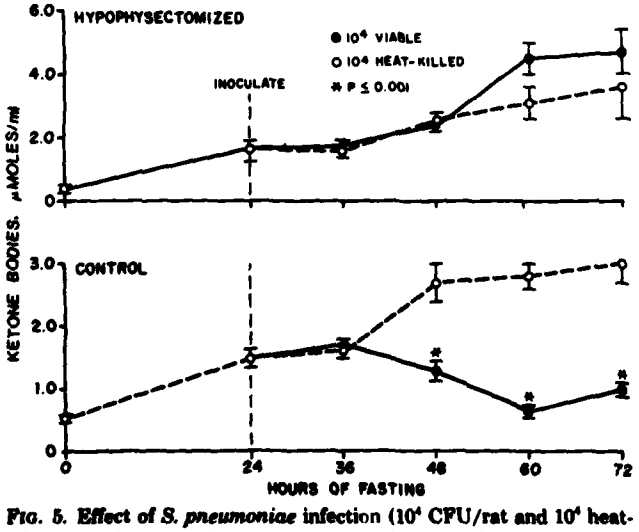

FIG. 5. Effect of *S. pneumoniae* infection (10^4 CFU/rat and 10^4 heat-killed organisms) on the concentration of plasma ketone bodies in hypophysectomized and normal rats.

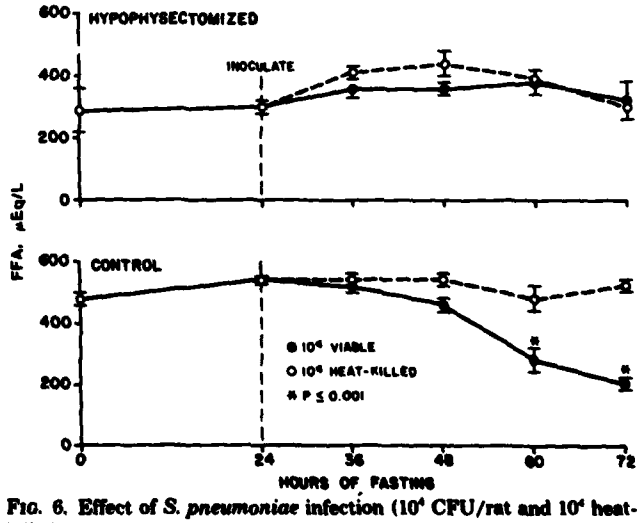

FIG. 6. Effect of *S. pneumoniae* infection (10^4 CFU/rat and 10^4 heat-killed organisms) on the concentration of plasma FFA in hypophysectomized and normal rats.

rat (26).

In rats with experimentally induced diabetic ketosis there are marked increases in plasma ketone and FFA concentrations, with a depressed insulin to glucagon ratio. These changes are not appreciably altered when infection is induced in an untreated diabetic rat. In several experiments, no appreciable variations in ketone bodies were noted during infection. Since the animals were so demonstrably ketotic, we did not consider a *P* value greater than 0.01 to be relevant; indeed, in other

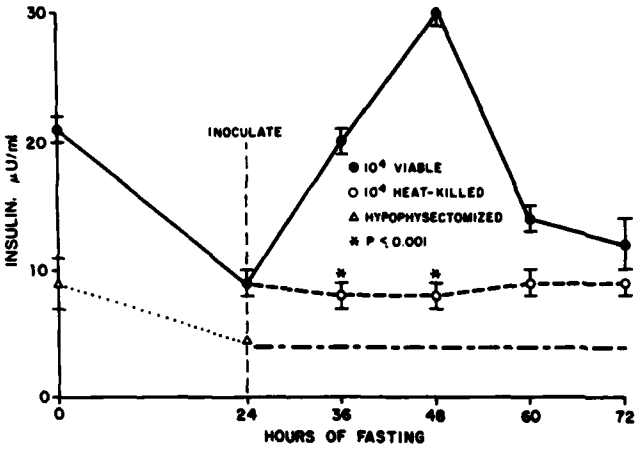

FIG. 7. Effect of *S. pneumoniae* infection [10^4 CFU/rat (●) and 10^4 heat-killed organisms (○)] on the level of plasma insulin in the hypophysectomized (△) and normal rats. The level of insulin in the fasted infected or uninfected hypophysectomized rats was below detectable limits (— - —).

experiments the *P* value was much greater than 0.05. Similarly, when hypophysectomized rats developed pneumococcal sepsis, they maintained a degree of starvation ketonemia similar to that observed in noninfected hypophysectomized rats. The absence of thyroid and adrenal glands had no detectable effect on the infection-related inhibition of starvation-induced ketonemia. The effect of infection in female rats was similar to that in males. Thus, thyroid, adrenal, or gonadal hormones do not appear to be responsible for the reduced ketosis during pneumococcal sepsis in the rat. However, the plasma concentration of insulin in the fasted infected or noninfected hypophysectomized rat was below the detectable level for the procedure employed. Further, pneumococcal sepsis in the diabetic rat did not result in inhibition of starvation-induced ketonemia. Thus, it has been hypothesized that the increased release of insulin during infection/inflammation could play a role in inhibiting starvation-induced ketonemia.

Recent data (27) have indicated that during caloric deprivation associated with severe sepsis, the general failure of ketonemic adaptation to starvation is the result of a reduced ketogenic capacity of the liver. Further, the decrease in plasma FFA content is associated with the reduced albumin content, so that the hepatic supply of FFA may not have been altered (28). Although the exact stimulus for activation of hepatic ketogenesis has not been elucidated, McGarry *et al.* (29) have shown that the *in vivo* injection of antiinsulin serum or glucagon rapidly increases the ketogenic capacity of the liver in the fed rat. This has led to the theory that hepatic ketogenesis is under bihormonal control, with glucagon being stimulatory and insulin inhibitory. Antagonism between the effects of insulin and glucagon has been demonstrated by Mackrell and Sokal (26). However, in a fasted rat, a direct inhibitory effect of insulin on the hepatic ketogenic capacity could not be demonstrated *in vitro*. This observation suggests an indirect role of insulin in inhibiting hepatic ketogenic capacity.[1]

During pneumococcal sepsis in the rat, both portal venous and inferior vena caval concentrations of immunoreactive glucagon and insulin are increased very early in infection, with a significant reduction in the insulin to glucagon ratio (30). Similar increases in plasma glucagon and insulin and decreases in the insulin to glucagon molar ratio have been observed during bacterial and viral infections in humans (31, 32) and monkeys (12). From this profile of glucagon and insulin, the ketogenic capacity of liver should be theoretically increased in the infected host. Instead, a decrease has been observed. At the present time, it can only be hypothesized that the increased plasma insulin concentration observed during infection/inflammation plays an indirect role in inhibiting hepatic ketogenic capacity by an as yet unresolved mechanism.

A number of investigators (32–38) have suggested that both neural and hormonal factors from the region of the hypophysis are involved in the stimulation of insulin and glucagon release from the pancreas. Our data do not yet allow the postulation of a mechanism to fully explain the factors governing the decrease in ketones accompanying an inflammatory stress. The lack of this response in hypophysectomized animals suggests that the inflammatory response causes the hypophysis, in an as yet unexplained manner, to affect the endocrine pancreas. The data do suggest an indirect role of insulin in the inhibition of starvation-induced ketosis during infection/inflammation in the rat.

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[1] In unpublished experiments, it has been demonstrated that perfusion of insulin has no effect on the ketogenic capacity of the isolated rat liver (Pace, J. G., personal communication).

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